2010

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Yeast
Yeast 2010; **27**: 389–398.
Published online 12 March 2010 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/yea.1763

Research Article

Yeasts associated with nectarines and their potential for biological control of brown rot†

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†*Mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.*

Received: 2 October 2009
Accepted: 17 January 2010

Abstract

Resident fruit microflora has been the source of biocontrol agents for the control of postharvest decay of fruits and the active ingredient in commercialized biocontrol products. With the exception of grapes and apples, information on the resident microflora of other fruits is only fragmentary, but greater knowledge in this area can be very helpful in developing biocontrol strategies. We characterized the yeast microflora of nectarines ('Croce del Sud') from the early stages of fruit development until harvest. The fruit samples were collected from trees in an unmanaged orchard. The resident fruit microflora was separated from the occasionally deposited microorganisms by discarding initial fruit washings before the final wash, followed by sonication and plating on NYDA medium. The isolated yeasts were identified by BIOLOG and by sequencing the D1/D2 domain of a large subunit of the rRNA gene and, where available, the ITS sequence. BIOLOG identified 19 and the genetic analysis 23 species of yeasts. Although the identification by these two systems was not always the same, the predominant yeasts were *Rhodotorula* spp., *Sporodiobolus* spp., *Cryptococcus* spp., *Pichia* spp., *Candida* spp. and yeast-like *Aureobasidium pullulans*. Several of the taxa appear to represent new species. The preliminary biocontrol tests against brown rot of nectarine fruit caused by *Monilinia fructicola* indicates significant decay control potential of some of the identified yeast species, namely *Cryptococcus magnus, Cryptococcus* sp. nov., *Sporidiobolus pararoseus, A. pullulans* and *Rhodotorula* sp. nov.

Keywords: yeast communities; fruit microflora; postharvest biocontrol; *Monilinia fructicola*

Introduction

Natural fruit surface microflora includes yeasts, bacteria and filamentous fungi. The growth of these microorganisms is supported by nutrients leaking from the fruit, as well as external sources, such as pollen deposits, a variety of organic debris and honeydew (Blakeman, 1985). These sources provide the food base for saprophytic microorganisms and can be utilized by parasities as well, before establishing parasitic interaction with the host. As the season progresses, the composition of the fruit surface microflora changes (Janisiewicz and Buyer, 2010). This may be due to flux in the nutritional status of the fruit, such as differences in abundance of carbon and nitrogen compounds (sugar and amino acids) and tissue pH. Under favourable climatic conditions, such as moderate temperatures and adequate moisture, microorganisms may flourish on the fruit surface. In biological control of postharvest diseases (BCPD) of fruit, the fruit microflora has been the main source of antagonists (Chand-Goyal and Spotts, 1996; Droby *et al*., 1999; Janisiewicz, 1987; Wilson *et al*., 1993; Usall

et al., 2000), some of which have been commercialized (Janisiewicz and Korsten, 2002). Antagonists selected from the resident fruit microflora may have ecological advantages over antagonists originating from microflora casually deposited on the fruit or those residing mainly in the soil. They may also be more acceptable as a postharvest fruit treatment to the public and regulatory agencies, since they have been consumed with fruit for millennia without known adverse effects.

In comparison to other plant parts, relatively little is known about the microbial ecology of fruit surfaces. Most of the work was done on grape and apple, often in relation to vine and apple cider production (Beech, 1993; Clark *et al*., 1954; Davenport 1976a, 1976b; Waida *et al*., 1983; Williams *et al*., 1956) and most recently on pome and citrus fruits in relation to biological control of fruit decays (Droby *et al*. 1999; Chand-Goyal and Spotts, 1996; Janisiewicz, 1987; Janisiewicz *et al*., 2001). Knowledge of microbial ecology of other fruits, including stone fruits, is often limited to reports on the presence of the individual microorganisms at a given time, without any attempt to determine their residential status or place in the microbial succession (Buhagiar and Barnett, 1971; Dennis, 1976; Sasaki and Yoshida, 1959; Stallarova, 1982). The natural microflora of plums and nectarines has not been characterized and explored for their biocontrol potential, and information about these microflora is limited to a description of a few yeast species on plums and cherries (Stollarova, 1982, 1984).

The main objective of this study was to describe the resident yeast microflora of intact nectarine fruit from the early stages of fruit development to maturity, and to determine their antagonistic potential against *Monilinia fructicola*, the causal agent of brown rot, which is a very destructive postharvest disease of stone fruits.

Materials and methods

Orchard and fruit sampling

Nectarine trees 'Croce del Sud' were grown in an unmanaged orchard at the Appalachian Fruit Research Station in Kearneysville, WV. The trees were 7 years old during the first year of isolation. Fruit samples for isolations were collected aseptically from five trees, four times during fruit development from early stages until maturity, in two consecutive years (2006 and 2007).

Microbe isolation

The fruit samples were washed in 500 ml beakers with phosphate buffer (0.05 M, at pH 6.8) by shaking on a rotary shaker for 3 min at 75 rpm. The washings were discarded, and fresh buffer was added to the beakers. The fruit was sonicated using a Branson 1510 sonication bath (Branson Ultrasonic Corp., Eagle Road, Danbury, CT, USA) for 1 min and washed again by shaking for 15 min at 75 rpm. These washings were dilution-plated on nutrient yeast dextrose agar (NYDA) medium (Difco, Becton-Dickinson, Sparks, MD, USA). The plates were incubated at 26 °C for up to 7 days and developing colonies were isolated randomly and based on different phenotypic characteristics. The plates were re-examined after 2 weeks of incubation at 4 °C and additional isolations were made. The colonies were purified by triple restreaking of single colonies and preserved in 15% glycerol at −80 °C. The code for the isolates reflected nectarine (Ne) as the source of isolation, the year of isolation 6 or 7 (for 2006 or 2007), the time of isolation [T1, 6 weeks before harvest (wbh); T2, 4 wbh; T3, 2 wbh; T4, harvest), the tree sample (S) and the isolate (I) number. The AFRS–Kearneysville and NRRL Collection (Peoria, IL, USA) codes are listed in Table 2.

Isolates identification

Purified isolates were differentiated as to bacteria, yeast, and filamentous fungi. The yeasts were identified using the BIOLOG ML 4.2 system (Biolog Inc., Hayward, CA, USA) and genetically, using the sequences of the D1/D2 domain of the large subunit (*LSU*) rRNA gene and the ITS sequence according to procedures described by Kurtzman and Robnett (1998, 2003).

Preparation of pathogen and yeast inocula

The pathogen, *Monilinia fructicola* (isolate Mf7) was isolated from a decayed peach and was maintained on peach agar medium (PA) under constant light at 22 °C. The PA medium contained 900g/l canned peach halves in heavy syrup, which were washed three times in sterile distilled water,

DOI: 10.1002/yea

blended in a blender and 22 g/l agar was added. Conidia were collected from 10–14 day-old sporulating cultures with a vacuum spore collector, suspended in 0.05% Tween 20, sonicated, vortexed and adjusted with water to a concentration of 10^5 conidia/ml, using a haemocytometer. Then, two 10-fold dilutions were made to obtain two additional concentrations of 10^4 and 10^3 conidia/ml, which were also used for fruit inoculation.

The yeast cultures were activated from stock cultures by transferring to plates with NYDA medium. After growing to easily visible culture, they were transferred again to NYDA medium, grown overnight, and the yeast suspensions were prepared by suspending the culture in sterile tap water and adjusting the concentration to 50% transmittance at 420 nm.

Biocontrol tests

Throughput screening of the yeast isolates was conducted on nectarine fruits. Harvested fruits were washed, randomized and placed on fruit pack trays in plastic boxes. The fruits were wounded in the middle with a cylindrical tool, 3 mm in diameter and 3 mm deep, and the cut tissue was removed; 25 µl of the aqueous antagonist suspension was placed in each wound, and after approximately 1.5 h the wounds were inoculated with 25 µl of a conidial suspension of *M. fructicola* at concentrations of 10^3, 10^4 or 10^5 conidia/ml. The fruits were incubated at 22 °C for 3 days and the lesion size was measured perpendicular to the stem axis. Each isolate was tested on nine fruits, three for each concentration of the pathogen.

Data analysis

BIOLOG data from individual trees and for samples collected during the season were combined and subjected to BIOLOG cluster analysis (program included in the BIOLOG MicroLog™ 3 System, Version 4.20.05, Biolog Inc., Hayward, CA, USA), and dendrograms expressing units of taxonomic distance were generated (see dendrograms at: **http://www.ars.usda.gov/pandp/docs.htm?docid=17505**). To compare the two identification methods, results from the genetic identification were listed, along with the BIOLOG identification, on the dendrograms. ITS sequences were aligned in CLC Sequence Viewer 6.1 (**http://www.clcbio.com**). A distance matrix was constructed from the aligned sequences using the Phylip program dnadist (**http://evolution.genetics.washington.edu/phylip.html**). The distance matrix was analysed and graphed by non-metric multidimensional scaling, using proc MDS in SAS (SAS Institute, Cary, NC, USA). ANOVA (proc GLM, SAS) was used to determine whether the coordinates generated by non-metric multidimensional scaling were significantly different at different sampling times. Species abundance tables (species or genus vs. sampling time) were constructed and statistically tested using proc FREQ (SAS). Fisher's exact test was conducted using the Monte Carlo estimation.

Results and discussion

Our results indicate that the genetic identification is more consistent and reliable than BIOLOG identification so all of our references to the identity of the isolates are based on genetic identification. Nineteen species were identified by BIOLOG (see data at: **http://www.ars.usda.gov/pandp/docs.htm?docid=17505**) and 23 species and 17 genera were identified by genetic methods (Tables 1, 2). The species identified by BIOLOG were often different from those identified genetically. Nevertheless, the BIOLOG grouping often reflected genetic identification, indicating a very limited library in the BIOLOG system. Cluster analysis of the BIOLOG data resulted in 11 major clusters that were complementary with genetic rather than BIOLOG identification. For example, one cluster had 17 isolates which represented only two species using genetic identification, while BIOLOG identified four species and only half of the isolates were identified.

Cryptococcus spp., *Aureobasidium* sp., *Rhodotorula* spp. and *Sporidiobolus* sp. were isolated throughout fruit development and together constituted 84.72% of all isolations (Table 1). Other species, such as *Erythrobasidium, Pseudeurotium* or *Tremella* were isolated during the early stage of fruit development, while *Hanseniaspora, Candida, Acremonium, Metschnikowia, Pichia, Sporobolomyces* and *Zygosaccharomyces* were isolated mainly from mature fruits. Some isolates were identified by the genetic methods only to genus, and their species identity awaits further investigation. Several of the taxa appear to represent new species.

DOI: 10.1002/yea

Table 1. Frequency analysis of the occurrence of yeast genera at various sampling times during a 2 year period based on the DNA ITS sequence distance data analysis

	Time of sampling				
Genus	**T1**[1]	**T2**	**T3**	**T4**	**Total**
Acremonium	0[2]	0	0	1	1
	0.00[3]	0.00	0.00	0.55	0.55
	0.00[4]	0.00	0.00	100.00	
	0.00[5]	0.00	0.00	2.94	
Aureobasidium	12	13	11	7	43
	6.59	7.14	6.04	3.85	23.63
	27.91	30.23	25.58	16.28	
	30.00	22.03	22.45	20.59	
Auriculibuller	2	0	1	0	3
	1.10	0.00	0.55	0.00	1.65
	66.7	0.00	33.33	0.00	
	5.00	0.00	2.04	0.00	
Candida	0	0	0	2	2
	0.00	0.00	0.00	1.10	1.10
	0.00	0.00	0.00	100.00	
	0.00	0.00	0.00	5.88	
Cryptococcus	9	17	14	8	48
	4.95	9.34	7.69	4.40	26.37
	18.75	35.42	29.17	16.67	
	22.5	28.81	28.57	23.53	
Dothichiza	0	0	1	0	1
	0.00	0.00	0.55	0.00	0.55
	0.00	0.00	100.00	0.00	
	0.00	0.00	2.04	0.00	
Erythrobasidium	1	5	0	0	6
	0.55	2.75	0.00	0.00	3.30
	16.67	83.33	0.00	0.00	
	2.5	8.47	0.00	0.00	
Hanseniaspora	0	0	0	1	1
	0.00	0.00	0.00	0.55	0.55
	0.00	0.00	0.00	100.00	
	0.00	0.00	0.00	2.94	
Metschnikowia	0	1	0	1	2
	0.00	0.55	0.00	0.55	1.10
	0.00	50.00	0.00	50.00	
	0.00	1.69	0.00	2.94	
Pichia	0	0	0	1	1
	0.00	0.00	0.00	0.55	0.55
	0.00	0.00	0.00	100.00	
	0.00	0.00	0.00	2.94	
Pseudoeurotium	1	0	0	0	1
	0.55	0.00	0.00	0.00	0.55
	100.00	0.00	0.00	0.00	
	2.5	0.00	0.00	0.00	
Rhodotorula	11	14	11	5	41
	6.04	7.69	6.04	2.75	22.53
	26.83	34.15	26.83	12.20	
	27.50	23.73	22.45	14.71	
Sporidiobolus	3	7	8	6	24
	1.65	3.85	4.40	3.30	13.19
	12.5	29.17	33.33	25.00	
	7.5	11.86	16.33	17.65	
Sporobolomyces	0	0	2	0	2
	0.00	0.00	1.10	0.00	1.10
	0.00	0.00	100.00	0.00	
	0.00	0.00	4.08	0.00	
Taphrina	1	1	1	1	4
	0.55	0.55	0.55	0.55	2.20
	25.00	25.00	25.00	25.00	
	2.50	2.50	2.50	2.50	
Tremella	0	1	0	0	1
	0.00	0.55	0.00	0.00	0.55
	0.00	100.00	0.00	0.00	
	0.00	1.69	0.00	0.00	
Zygosaccharomyces	0	0	0	1	1
	0.00	0.00	0.00	0.55	0.55
	0.00	0.00	0.00	100.00	
	0.00	0.00	0.00	2.94	
Number of isolates	40	59	49	34	182
Percentage of total isolates	21.98	32.42	26.92	18.68	100.00

[1] Time of sampling: T1, 6 weeks before harvest (wbh); T2, 4 wbh; T3, 2 wbh; T4, harvest.
[2] Frequency of occurrence (number of isolates) of the genera at a sampling time.
[3] Percentage of a genus in relation to all isolates of all genera from all sampling times.
[4] Percentage of a genus in relation to this genus at various sampling times.
[5] Percentage of a genus in relation to all genera at a given sampling time.

Non-metric multidimensional scaling was used to graphically represent the yeast community (Figure 1a). The final badness of fit criterion, equivalent to Kruskal's stress formula 1, was 0.120, which is considered satisfactory for ecological data (McCune and Grace, 2002). While the communities in each of the first three sampling times were not differentiated from each other by this analysis, the community of the fourth sampling time partially separated from the other three (Figure 1b). The separation of sampling 4 from the first three samplings was statistically significant for dimension 2 in 2006 ($p = 0.0001$) but not in 2007. The effect of sampling time on the distribution of isolates within genera was further analysed by frequency analysis (Table 1). The probability of no

DOI: 10.1002/yea

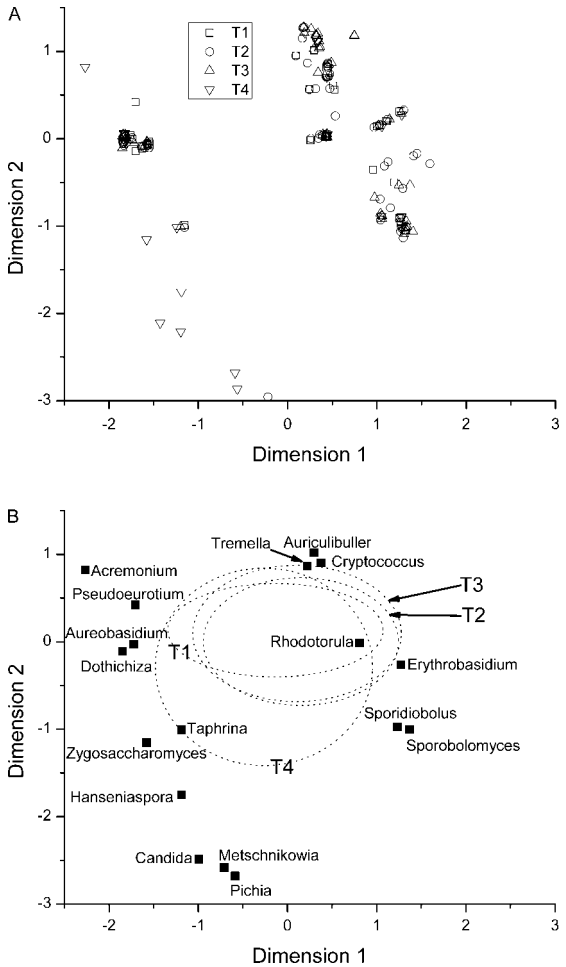

Figure 1. Non-metric multidimensional scaling of distance matrix of ITS sequence data of yeasts. (A) All samples from both years were plotted; squares, T1; circles, T2; triangles, T3; inverted triangles, T4. (B) The means for each genus were plotted as points, while the means and standard deviations for each sampling time were plotted as ellipses

association between genus and time was 1×10^{-20}, indicating a statistically significant effect of time on genus.

In contrast to yeast, bacterial communities isolated at the same time and from the same trees as the yeast in this study were significantly different from each other at each time point (Janisiewicz and Buyer, 2010). While the analytical and statistical methods used for bacteria and yeasts were different, it still appears that yeast succession at the species and genus levels was much less pronounced than bacterial succession. This may result from better adaptation to the changing fruit environment by the few yeasts, which was demonstrated by their dominance throughout the growing season.

Three out of four of the most predominant yeasts, *Cryptococcus* spp., *Aureobasidium* sp. and *Rhodotorula* spp., have been reported to be effective biocontrol agents against a number of postharvest diseases of various fruits (Calvente, 2001; Ippolito and Nigro, 2000; Leibinger *et al*., 1997; Lima *et al*. 1998; Roberts, 1990; Zhang *et al*., 2008). *Aureobasidium* was also reported to control field diseases (Dimakopoulus *et al*., 2008; Kunz, 2004; Lima *et al*. 1997). This may result from the ability to colonize fruits throughout the growing season. This is in contrast to yeasts isolated from fruit at the most mature stage, such as *Zygosaccharomyces bailii* or *Hanseniaspora uvarum*, which could be explored for postharvest protection. However, their usefulness must be carefully considered, as they may be involved in the spoilage of fruit juices. In research on developing biocontrol of field diseases of stone fruit, it may also be advantageous to focus on *Cryptococcus* spp., *Rhodotorula* spp. and *Sporidiobolus* sp. as they also colonize fruit throughout the growing season.

Results from the biocontrol test on controlling infections originating from wounds after harvest indicate a significant potential of some of these yeasts for controlling infections originating from wounds (Table 3). There was a wide range of effectiveness, even between isolates within a species. The most effective yeasts were *Cryptococcus magnus, Cryptococcus* sp. nov., *Aureobasidium pullulans, Rhodotorural sp. nov.* and *Sporidiobolus papraroseus*. However, more research is needed to determine their usefulness for practical applications against brown rot.

This is the first report describing residential populations of yeasts on nectarine fruit. Our long-term goal is to develop postharvest biological control of brown rot originating from latent infection by *M. fructicola* occurring in the field, using naturally occurring antagonists. The ability to control latent infections may be different from protecting wounds, as the most critical part of the mechanism of biocontrol for latent infections is the direct colonization of the pathogen appressoria. Our current research is focused on developing methods for screening yeasts against latent infections, using both *in vitro* and *in situ* systems.

DOI: 10.1002/yea

Table 2. Identity of yeast isolates from nectarine fruit. Identifications are based on a BLAST search of GenBank using the D1/D2 sequences of the large subunit rRNA gene and, where available, confirmed with the ITS sequence

NRRL[1] code	Kearneysville code	Isolate identification
NRRL Y-48335	Ne6T4-S1I5	*Acremonium strictum* or related species
NRRL Y-48493	Ne7T1-S1I1	*Aureobasidium pullulans*[2]
NRRL Y-48494	Ne7T1-S1I7	*Aureobasidium pullulans*
NRRL Y-48496	Ne7T1-S1I20	*Aureobasidium pullulans*
NRRL Y-48498	Ne7T1-S2I7	*Aureobasidium pullulans*
NRRL Y-48505	Ne7T1-S4I5	*Aureobasidium pullulans*
NRRL Y-48510	Ne7T1-S5I5	*Aureobasidium pullulans*
NRRL Y-48520	Ne7T2-S2I9	*Aureobasidium pullulans*
NRRL Y-48524	Ne7T2-S3I5	*Aureobasidium pullulans*
NRRL Y-48532	Ne7T2-S4I6	*Aureobasidium pullulans*
NRRL Y-48542	Ne7T2-S5I18	*Aureobasidium pullulans*
NRRL Y-48545	Ne7T3-S1I7	*Aureobasidium pullulans*
NRRL Y-48548	Ne7T3-S1I11b	*Aureobasidium pullulans*
NRRL Y-48550	Ne7T3-S2I1	*Aureobasidium pullulans*
NRRL Y-48554	Ne7T3-S2I7	*Aureobasidium pullulans*
NRRL Y-48559	Ne7T3-S3I2	*Aureobasidium pullulans*
NRRL Y-48567	Ne7T3-S4I3	*Aureobasidium pullulans*
NRRL Y-48570	Ne7T3-S5I7	*Aureobasidium pullulans*
NRRL Y-48579	Ne7T4-S1I9	*Aureobasidium pullulans*
NRRL Y-48587	Ne7T4-S2I4	*Aureobasidium pullulans*
NRRL Y-48588	Ne7T4-S2I5	*Aureobasidium pullulans*
NRRL Y-48594	Ne7T4-S3I8	*Aureobasidium pullulans*
NRRL Y-48596	Ne7T4-S4I1	*Aureobasidium pullulans*
NRRL Y-48599	Ne7T4-S5I4	*Aureobasidium pullulans*
NRRL Y-48272	Ne6T1-S2I4	*Aureobasidium pullulans*
NRRL Y-48273	Ne6T1-S2I5	*Aureobasidium pullulans*
NRRL Y-48274	Ne6T1-S4I10	*Aureobasidium pullulans*
NRRL Y-48276	Ne6T1-S5I2	*Aureobasidium pullulans*
NRRL Y-48278	Ne6T1-S1I3	*Aureobasidium pullulans*
NRRL Y-48289	Ne6T1-S5I4	*Aureobasidium pullulans*
NRRL Y-48291	Ne6T2-S1I8	*Aureobasidium pullulans*
NRRL Y-48292	Ne6T2-S1I9b	*Aureobasidium pullulans*
NRRL Y-48293	Ne6T2-S1I11	*Aureobasidium pullulans*
NRRL Y-48298	Ne6T2-S2I4	*Aureobasidium pullulans*
NRRL Y-48304	Ne6T2-S3I3	*Aureobasidium pullulans*
NRRL Y-48307	Ne6T2-S3I10	*Aureobasidium pullulans*
NRRL Y-48311	Ne6T2-S4I2	*Aureobasidium pullulans*
NRRL Y-48319	Ne6T2-S5I6b	*Aureobasidium pullulans*
NRRL Y-48323	Ne6T2-S5I11	*Aureobasidium pullulans*
NRRL Y-48327	Ne6T3-S1I9	*Aureobasidium pullulans*
NRRL Y-48329	Ne6T3-S2I7	*Aureobasidium pullulans*
NRRL Y-48331	Ne6T3-S3I7	*Aureobasidium pullulans*
NRRL Y-48334	Ne6T3-S5I8	*Aureobasidium pullulans*
NRRL Y-48336	Ne6T4-S1I6	*Aureobasidium pullulans*
NRRL Y-48512	Ne7T1-S5I9a	*Auriculibuller fuscus*
NRRL Y-48514	Ne7T1-S5I14	*Auriculibuller fuscus*
NRRL Y-48572	Ne7T3-S5I11	*Auriculibuller fuscus*
NRRL Y-48340	Ne6T4-S3I10	*Candida* sp. nov. 1
NRRL Y-48342	Ne6T4-S4I2	*Candida* sp. nov. 2
NRRL Y-48543	Ne7T3-S1I3	Crypt VKMY2958
NRRL Y-48544	Ne7T3-S1I5	Crypt VKMY2958
NRRL Y-48546	Ne7T3-S1I8	Crypt VKMY2958
NRRL Y-48591	Ne7T4-S3I3	Crypt VKMY2958
NRRL Y-48275	Ne6T1-S4I5	*Cryptococcus magnus*
NRRL Y-48314	Ne6T3-S4I6	*Cryptococcus magnus*
NRRL Y-48317	Ne6T2-S4I11	*Cryptococcus magnus*

Table 2. Continued

NRRL[1] code	Kearneysville code	Isolate identification
NRRL Y-48539	Ne7T2-S5I1	*Cryptococcus* sp. nov.
NRRL Y-48540	Ne7T2-S5I3	*Cryptococcus* sp. nov.
NRRL Y-48566	Ne7T3-S4I1a	*Cryptococcus* sp. nov.
NRRL Y-48574	Ne7T3-S5I3	*Cryptococcus* sp. nov.
NRRL Y-48590	Ne7T4-S2I8	*Cryptococcus* sp. nov.
NRRL Y-48282	Ne6T1-S2I1	*Cryptococcus* sp. nov.
NRRL Y-48301	Ne6T2-S2I1	*Cryptococcus* sp. nov.
NRRL Y-48312	Ne6T2-S4I5	*Cryptococcus* sp. nov.
NRRL Y-48320	Ne6T2-S5I8	*Cryptococcus* sp. nov.
NRRL Y-48326	Ne6T3-S1I8	*Cryptococcus* sp. nov.
NRRL Y-48553	Ne7T3-S2I6	*Cryptococcus victoriae*
NRRL Y-48562	Ne7T3-S3I8	*Cryptococcus victoriae*
NRRL Y-48497	Ne7T1-S2I4	*Cryptococcus weiringae*
NRRL Y-48500	Ne7T1-S2I14	*Cryptococcus weiringae*
NRRL Y-48502	Ne7T1-S3I14	*Cryptococcus weiringae*
NRRL Y-48506	Ne7T1-S4I9b	*Cryptococcus weiringae*
NRRL Y-48508	Ne7T1-S4I18	*Cryptococcus weiringae*
NRRL Y-48515	Ne7T2-S1I1	*Cryptococcus weiringae*
NRRL Y-48516	Ne7T2-S1I2	*Cryptococcus weiringae*
NRRL Y-48517	Ne7T2-S2I1	*Cryptococcus weiringae*
NRRL Y-48518	Ne7T2-S2I2	*Cryptococcus weiringae*
NRRL Y-48519	Ne7T2-S2I3	*Cryptococcus weiringae*
NRRL Y-48531	Ne7T2-S4I5	*Cryptococcus weiringae*
NRRL Y-48533	Ne7T2-S4I12	*Cryptococcus weiringae*
NRRL Y-48536	Ne7T2-S5I1a	*Cryptococcus weiringae*
NRRL Y-48537	Ne7T2-S5I4	*Cryptococcus weiringae*
NRRL Y-48577	Ne7T4-S1I1	*Cryptococcus weiringae*
NRRL Y-48584	Ne7T4-S2I1	*Cryptococcus weiringae*
NRRL Y-48585	Ne7T4-S2I2	*Cryptococcus weiringae*
NRRL Y-48271	Ne6T1-S1I2	*Cryptococcus wieringae*
NRRL Y-48277	Ne6T1-S5I3	*Cryptococcus wieringae*
NRRL Y-48297	Ne6T2-S2I3	*Cryptococcus wieringae*
NRRL Y-48306	Ne6T2-S3I9	*Cryptococcus wieringae*
NRRL Y-48324	Ne6T3-S1I1	*Cryptococcus wieringae*
NRRL Y-48325	Ne6T3-S1I3	*Cryptococcus wieringae*
NRRL Y-48557	Ne7T3-S2I13	*Cryptococcus* sp. nov.
NRRL Y-48563	Ne7T3-S3I10	*Cryptococcus* sp. nov.
NRRL Y-48575	Ne7T3-S5I17	*Cryptococcus* sp. nov.
NRRL Y-48583	Ne7T4-S1I17	*Cryptococcus* sp. nov.
NRRL Y-48595	Ne7T4-S3I9	*Cryptococcus* sp. nov.
NRRL Y-48601	Ne7T4-S5I12	*Cryptococcus* sp. nov.
NRRL Y-48560	Ne7T3-S3I4	*Dothichiza pithyophila*
NRRL Y-48279	Ne6T1-S1I4	*Erythrobasidium hasegawianum*
NRRL Y-48295	Ne6T2-S1I14	*Erythrobasidium hasegawianum*
NRRL Y-48300	Ne6T2-S2I9	*Erythrobasidium hasegawianum*
NRRL Y-48305	Ne6T2-S3I5b	*Erythrobasidium hasegawianum*
NRRL Y-48310	Ne6T2-S4I1	*Erythrobasidium hasegawianum*
NRRL Y-48338	Ne6T4-S2I5	*Hanseniaspora uvarum*
NRRL Y-48589	Ne7T4-S2I7	*Metschnikowia fructicola/pulcherrima*
NRRL Y-48529	Ne7T2-S4I3	near *Metschnikowia kunwiensis*
NRRL Y-48507	Ne7T1-S4I17	near *Pseudeurotium zonatim*
NRRL Y-48534	Ne7T2-S4I14	near *Rhodotorula philya*
NRRL Y-48576	Ne7T3-S5I18	near *Rhodotorula philya*
NRRL Y-48344	Ne6T4-S4I7	*Pichia kluyveri*
NRRL Y-48549	Ne7T3-S1I12	*Rhodotorula philya*
NRRL Y-48287	Ne6T1-S4I6a	*Rhodotorula glutinis*

DOI: 10.1002/yea

Table 2. Continued

NRRL[1] code	Kearneysville code	Isolate identification
NRRL Y-48290	Ne6T2-S1I4	*Rhodotorula glutinis*
NRRL Y-48308	Ne6T2-S3I12	*Rhodotorula glutinis*
NRRL Y-48328	Ne6T3-S2I4	*Rhodotorula glutinis*
NRRL Y-48495	Ne7T1-S1I17	*Rhodotorula hinnulea*
NRRL Y-48501	Ne7T1-S2I18	*Rhodotorula hinnulea*
NRRL Y-48525	Ne7T2-S3I7	*Rhodotorula hinnulea*
NRRL Y-48527	Ne7T2-S3I15	*Rhodotorula hinnulea*
NRRL Y-48528	Ne7T2-S3I16	*Rhodotorula hinnulea*
NRRL Y-48535	Ne7T2-S4I15	*Rhodotorula hinnulea*
NRRL Y-48541	Ne7T2-S5I17	*Rhodotorula hinnulea*
NRRL Y-48556	Ne7T3-S2I11	*Rhodotorula hinnulea*
NRRL Y-48561	Ne7T3-S3I6b	*Rhodotorula hinnulea*
NRRL Y-48565	Ne7T3-S3I12	*Rhodotorula hinnulea*
NRRL Y-48569	Ne7T3-S4I11	*Rhodotorula hinnulea*
NRRL Y-48573	Ne7T3-S5I12	*Rhodotorula hinnulea*
NRRL Y-48580	Ne7T4-S1I14	*Rhodotorula hinnulea*
NRRL Y-48582	Ne7T4-S1I16	*Rhodotorula hinnulea*
NRRL Y-48597	Ne7T4-S4I12	*Rhodotorula hinnulea*
NRRL Y-48280	Ne6T1-S2I2	*Rhodotorula hinnulea* or undescribed sister species
NRRL Y-48284	Ne6T1-S3I5	*Rhodotorula hinnulea* or undescribed sister species
NRRL Y-48309	Ne6T2-S3I14	*Rhodotorula hinnulea* or undescribed sister species
NRRL Y-48322	Ne6T2-S5I10	*Rhodotorula hinnulea* or undescribed sister species
NRRL Y-48503	Ne7T1-S3I17	*Rhodotorula pinicola*
NRRL Y-48513	Ne7T1-S5I12	*Rhodotorula pinicola*
NRRL Y-48522	Ne7T2-S2I14	*Rhodotorula pinicola*
NRRL Y-48558	Ne7T3-S2I15	*Rhodotorula pinicola*
NRRL Y-48581	Ne7T4-S1I15	*Rhodotorula pinicola*
NRRL Y-48281	Ne6T1-S2I10	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48283	Ne6T1-S3I4	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48286	Ne6T1-S4I3	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48288	Ne6T1-S4I8	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48296	Ne6T2-S1I15	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48302	Ne6T2-S2I12	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48315	Ne6T3-S4I7	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48316	Ne6T2-S4I10	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48332	Ne6T3-S3I11	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48337	Ne6T4-S2I3	*Rhodotorula* sp. near *Rhodotorula laryngis*
NRRL Y-48499	Ne7T1-S2I10	*Sporidiobolus pararoseus*
NRRL Y-48509	Ne7T1-S5I4	*Sporidiobolus pararoseus*
NRRL Y-48511	Ne7T1-S5I8b	*Sporidiobolus pararoseus*
NRRL Y-48521	Ne7T2-S2I10	*Sporidiobolus pararoseus*
NRRL Y-48530	Ne7T2-S4I4	*Sporidiobolus pararoseus*
NRRL Y-48538	Ne7T2-S5I5	*Sporidiobolus pararoseus*
NRRL Y-48547	Ne7T3-S1I11a	*Sporidiobolus pararoseus*
NRRL Y-48551	Ne7T3-S2I3	*Sporidiobolus pararoseus*
NRRL Y-48568	Ne7T3-S4I4a	*Sporidiobolus pararoseus*
NRRL Y-48571	Ne7T3-S5I9b	*Sporidiobolus pararoseus*
NRRL Y-48586	Ne7T4-S2I3	*Sporidiobolus pararoseus*
NRRL Y-48592	Ne7T4-S3I6	*Sporidiobolus pararoseus*
NRRL Y-48593	Ne7T4-S3I7	*Sporidiobolus pararoseus*
NRRL Y-48598	Ne7T4-S5I3	*Sporidiobolus pararoseus*
NRRL Y-48600	Ne7T4-S5I6	*Sporidiobolus pararoseus*
NRRL Y-48299	Ne6T2-S2I8	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48303	Ne6T2-S3I1	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48313	Ne6T3-S3I10	*Sporidiobolus pararoseus*—probable undescribed sister species

DOI: 10.1002/yea

Table 2. Continued

NRRL[1] code	Kearneysville code	Isolate identification
NRRL Y-48318	Ne6T2-S5I6a	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48330	Ne6T3-S2I10	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48333	Ne6T3-S4I8	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48339	Ne6T4-S3I6	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48354	Ne6T2-S1I12	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48355	Ne6T3-S3I8	*Sporidiobolus pararoseus*—probable undescribed sister species
NRRL Y-48555	Ne7T3-S2I8	*Sporobolomyces phaffi*
NRRL Y-48552	Ne7T3-S2I5	*Sporobolomyces roseus*
NRRL Y-48504	Ne7T1-S3I20	*Taphrina carnea*
NRRL Y-48564	Ne7T3-S3I11	*Taphrina carnea*
NRRL Y-48523	Ne7T2-S2I17	*Taphrina deformans*
NRRL Y-48578	Ne7T4-S16	*Taphrina deformans*
NRRL Y-48294	Ne6T2-S1I13	*Tremella globispora* or a sister species
NRRL Y-48343	Ne6T4-S4I6	*Zygosaccharomyces bailii*

[1] National Center for Agricultural Utilization Research (ARS Culture Collection, Pioria, IL, USA).
[2] Differed by two Ns and three indels in LSU D1/D2 from reference AFTOL-ID912, GenBank DQ470956. 0 diff. in ITS with EF690466. There is no living ex-type for *A. pullulans*, so the interpretation is that the following strains are *A. pullulans* or a closely related species.

Table 3. Bioconrol potential of yeasts isolated from nectarine fruit at various times against wound infection by *Monilinia fructicola* causing brown rot of stone fruits

Biocontrol category (% wounds infected)	Yeasts in each category[1] (%)
0	2.2
<34	23.0
34–66	36.3
66–100	38.5

[1] Results from screening on the fruit inoculated with the highest (10^5 conidia/ml) concentration of *M. fructicola*.

Acknowledgements

We would like to thank Roger Lewis and Eleanor Basehoar-Powers for their technical assistance in this project.

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